PROTECTIVE EFFECT OF *RUMEX HASTATUS* LEAVES EXTRACT AGAINST HEPATIC AND COGNITIVE DECLINE IN ANIMAL MODELS OF LIVER CIRRHOSIS.

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Protective effect of *Rumex hastatus* leaves extract against hepatic and cognitive decline in animal models of liver cirrhosis**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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INTRODUCTION

Liver is the main organ of metabolism and energy production. Its other main functions include: bile production, storage of iron, vitamins and trace elements, detoxification, conversion of waste products for excretion by the kidneys.

The liver is functionally divided into two lobes, right and left. The external division is marked on the front of the liver by the falciform ligament, which joins the coronary ligament at the superior margin of the liver. It receives 30% of the resting cardiac output and acts as a giant chemical processing plant in the body. These chemical reactions, called metabolism, are central in the regulation of body homeostasis. It also stores nutrients and other useful substances, as well as detoxifying or breaking down harmful compounds. These can then be excreted from the body in bile via the liver; in urine via the kidney, or by other means. The liver metabolises both beneficial and harmful substances. It has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Thus, the liver is subjected to a variety of diseases and disorders (which may be acute or chronic) caused by these agents or their metabolites. Acute and chronic liver diseases constitute a global concern, but medical treatments for these diseases are often difficult to handle and have limited efficacy (Kim *et al.*, 2009).

The liver performs a variety of important host defense and metabolic functions that include synthesis of acute-phase proteins, gluconeogenesis, detoxification, and clearance of endogenous mediators, as well as secretion of pro-inflammatory cytokines (Pastor *et al.*, 1995). It is a unique organ in the sense that a significant loss of liver cells due to drug toxicity or other insults can be overcome by regeneration (Mehendale, 2005).

It is reported that patients with chronic liver disease and liver cirrhosis are at increased risk for a wide spectrum of neurological complications (Ferro JM, 2014). These neurological complaints can range from subtle neuropsychiatric symptoms such as behavioral changes, abnormality of movements to overt hepatic encephalopathy. Cirrhosis-associated neurological co morbidities also include depression, bipolar disorder, schizophrenia, substance abuse and suicidal tendencies. Broadly, form a neuro-radiological perspective the neurological complications associated with chronic liver disease can be divided into direct effects of cirrhosis on the nervous system and neurological complications related to specific etiologies of liver disease (for e.g. alcohol, hepatitis C infection, Wilson disease) (White H, 2014).

Cirrhosis produces a wide spectrum of complications from portal hypertension, bleeding and bacterial infections to neurological disorders related with hyperammonaemia and brain oedema, which play a central role in the pathogenesis of hepatic encephalopathy (HE) (Butterworth RF, 2003, Olde Damink SW, 2002).

Hepatic Encephalopathy [HE] refers to a complex, potentially reversible or progressive syndrome of cerebral dysfunction, which consists of neuropsychiatric, cognitive and motor disturbances, characterized by a broad etiological spectrum. Hepatic encephalopathy (HE) is a neuropsychiatric disorder or loss of brain function occurs when the liver is unable to remove toxins from the blood that results from impaired liver function. This causes a buildup of toxins in the bloodstream, which can lead to brain damage. Hepatic encephalopathy can be acute (short-term) or chronic (long-term). In some cases, a person with hepatic encephalopathy may become unresponsive and slip into a coma (Ferenci P, 1998, Vilstrup H, 2014).

Acute hepatic encephalopathy develops because of severe liver disease. This condition mainly occurs in people with:

- acute fulminant viral hepatitis: a severe type of viral hepatitis that comes on suddenly
- toxic hepatitis: may be caused by exposure to alcohol, chemicals, drugs (recreational or prescription), or supplements
- Reye's syndrome: a rare and serious condition primarily seen in children that causes sudden swelling and inflammation of the liver and brain

Chronic hepatic encephalopathy may be permanent or recurrent. Those with the recurrent version will have multiple episodes of hepatic encephalopathy throughout their lives. They will also require continuous treatment to help prevent the development of symptoms. Recurrent cases are usually seen in people with severe cirrhosis (scarring of the liver).

In humans, HE occurs in one of the three forms that have recently been reclassified. Classification of hepatic encephalopathy syndromes

1. Type A Hepatic encephalopathy (HE) associated with acute liver failure (ALF) resulting from severe inflammatory and/or necrotic liver disease of rapid onset.
2. Type B HE resulting from portacaval shunting in the absence of parenchymal liver disease.
3. Type C HE that accompanies chronic liver failure (cirrhosis). Aetiology of cirrhosis resulting from alcohol, viral infection, biliary obstruction, drugs or toxins. Portal hypertension arises as a result of cirrhosis; the high portal pressure stimulates the opening of

embryonic venous channels. Portal-systemic shunts then permit toxins of intestinal origin (examples include ammonia, manganese, cytokines) to bypass the liver into the systemic circulation.

Hepatic encephalopathy may be triggered by:

- Infections such as pneumonia
- kidney problems
- dehydration
- low oxygen levels (hypoxia)
- recent surgery or trauma
- use of medications to suppress the immune system
- eating too much protein
- use of medications (such as barbiturates or benzodiazepine tranquilizers) that suppress the central nervous system
- electrolyte imbalance, especially a decrease in potassium after vomiting or taking diuretics

Central nervous system (CNS) functions are often affected secondary to liver damage. This effect is particularly observed on cognition and memory **(Gilberstadt et al., 1980).**

Pathogenesis of HE is still not well understood; one of the main hypothesis suggests a state of hyperammonemia, leading to both direct and indirect alterations in cerebral metabolism **(Rose C, 2002).** Ammonia is a product of protein degradation. At high concentrations ammonia leads to impaired cerebral function **(Plum, 1971; Duffy & Plum, 1982; Felipo & Butterwoth, 2002).** In liver disease (e.g. in liver cirrhosis) ammonia and other toxic substances are not properly detoxified, reaching the brain and altering its function, resulting in hepatic encephalopathy and intellectual impairment.

Hyperammonemia results in increased levels of glutamine in astrocytes from amidation of glutamate by glutamine synthetase **(Butterworth RF, 2002)** producing osmotic stress and swelling of astrocytes, leading to cerebral edema and intracranial hypertension which are ascribed to be the main pathophysiological features of HE **(Shawcross D, 2005).** Therefore, it is important to unravel the mechanisms by which the factors associated with liver failure affect cerebral function and animal models are of relevance in the investigation of underlying mechanisms of HE.

HE could be mediated by increased interleukin 1β (IL-1β) in hippocampus that triggers other pathways including the GABA and release of other cytokines (IL-4, IL-10 and TNF-α). These releasing of cytokines effects were associated with the activation of nuclear factor-kappa B (NF- -kB) activation. Furthermore, elevated expression of IL-1β in hippocampus has been associated with

impairment of spatial learning and memory **(Moore AH, 2009).** A main mechanism by which over activation of IL-1β receptor in hippocampus leads to cognitive impairment is by enhancing GABAergic neurotransmission. **Wang et al. 2012** showed active role of IL-1β to switch the membrane expression of $GABA_A$ receptors and of an associated inhibitory current in hippocampus and impaired learning, through activation of p38 mitogen activated protein kinase (MAPK). The previous studies suggest that impaired spatial learning could be due to neuroinflammation in hippocampus and that reducing it could restore spatial learning. Animals with experimentally induced acute liver failure show similar increases in blood-brain barrier permeability **(Zaki AE, 1984).**

Clinically, HE is one of the main causes of death in patients with liver disease, so early diagnosis and treatment are vital for better prognosis and improved survival rate of the HE patients (Li N, 2012).

In general, investigators have concentrated on behavioral study in hepatic damage only when the hepatic toxicant was administered in multiple and high doses to produce hepatic encephalopathy **(Shimomura et al., 1992; Baraldi et al., 1995)**, although behavioral and psychiatric deficits are some of the earliest signs in cirrhotic patients **(Watanabe et al., 1995).** The present investigation was designed to evaluate the protective effect of *Rumex hastatus* leave extract against hepatic and cognitive decline in animal models of liver cirrhosis i.e. Ethanol and Thioacetamide (TAA) induced hepatotoxicity in rats. The aim of this investigation is also to determine whether Ethanol and TAA could produce any behavioral impairment in rats. The effect of *Rumex hastatus* leave extract on the possible behavioral alterations secondary to liver damage produced by Ethanol and TAA in rats will also evaluate.

LITERATURE REVIEW

Plant under Investigation

Plant: *Rumex hastatus* Linn.

Family: : Polygonaceae (Buckwheat Family)

Taxonomic Position:

Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Polygonales
Family:	Polygonaceae
Genus:	*Rumex*
Species:	***Rumex hastatus***

Vernacular names:

English : Arrowleaf Dock, Yellow Sock, Curled sock

Hindi : Churki

Kumaon : Amlora, Chulmora

Nepali **:** Kapu, Charimaal

Punjab : Khattimal, Katamba

Urdu : Khatti Buti

Chemical constituents of *Rumex hastatus:*

The preliminary phytochemical screening of the ethanolic extract from the roots of *Rumex hastatus* (EERH) results with the presence of anthraquinone glycoside, tannins, flavonoids etc **(Khetwal et al., 1987; Tiwari and Sinha, 1980).** The levels of total phenols, tannins or condensed tannins were substantially lower for the young plant and the dead fallen stem than those for the mature plant. **(Makkar H.P.S. et al., 1993).** Two New Phenolic Glucosides from *Rumex hastatus* obtained were Hastatusides A and B **(Lan-Sheng Zhang).** The root and

bark yield 21–23% tannin **(Khare C.P., Indian medicinal plant)**. During search for bioactive metabolites from Rumex plants have reported several anthraquinones, naphthalenes, flavonoids nepalin, nepodin and rumicin and other phenolic compounds from *R. hastatus* **(Mei et al., 2009; Zhang et al., 2009, Nadkarni, 1976)**.

Origin and geographic distribution

Rumex hastatus is an annual, biennial and perennial herbs in the buckwheat family Polygonaceae. Members of this family are very common perennial herbs growing in acidic, sour soils mainly in the northern hemisphere. In India it is widely distributed in Himalayas, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, and Kumaun **(Shakuntala et al.)**

Medicinal uses of *R. hastatus*

- Roots of *Rumex hastatus* have grate mediation value are used in Ayurveda and other folk medicines for the treatment of different diseases and disorder such as rheumatoid arthritis, diarrhoea, dysenrty, diuretic, wound healing, neoplasm, jaundice etc. ***(*Coburn (1984) and Phole (1990) Pande *et al., 2007* and Zabta *et al, 2003)*.**
- The roots of *R. hastatus* are of medicinal significance for cough, headache, and fever **(Editorial Commission of China, 1998).**
- Juice of the root (about 2 teaspoons 3 times a day) is given to relieve diarrhoea and dysentery of a child. **(Narayan P. Manandhar, 1995).**
- Leaves are rubbed by the locals against stings by *Urtica dioica*.(**Mahroof Khan et al.,2009)**
- The juice of the plant is considered cooling, aperient and to a certain extent diuretic. (**Sheikh Saeed Ahmad,2007)**
- Fresh leaves are crushed along with water and sugar. One cup of this extract is given to the patient twice a day for two weeks used locally to treat jaundice and hepatitis **(Nadkarni KM, 1976).**
- *Rumex hastatus* juice is astringent and is used in bloody dysentery. The fresh tube is chewed to relieve throat aches. Root is laxative, alternative tonic and antirheumatic and is used in skin disease **(Manandhar, 2002).** The leaves and shoots of *R. hastatus* are diuretic, refrigerant and used as cooling agent **(Islam et al., 2006).**

- The leaves and shoots of *Rumex hastatus* and *Rumex dentatus* are diuretic, refrigerant and used as cooling agent **(Islam et al., 2006; Hussain et al., 2006).**

Reported Activities:

- Preliminary phytochemical screening, acute toxicity study and anti-diarrhoeal activity were studied on castor oil induced diarrhoea and normal gastrointestinal models of rats. **(Shakuntala et al.,2011)**.
- The methanol extract of the leaves of *R. hastatus* showed low antibacterial activity against *S. typhi*, *E. coli*, *S. aureus*, *P. aeruginosa* and *E. aerogenes* and highest antibacterial activity against *C. freundii.(* **Farrukh Hussain et al.2010).**
- The different solvent fractions (n-hexane, ethyl acetate, chloroform, butanol and aqueous) of the methanol extract of *Rumex hastatus*(RH) leaves were evaluated for antioxidant activities.**(Sumaira Sahreen et al., 2011)**
- The effect of methanolic extract of *Rumex hastatus* roots (MRR) and its derived fractions, n-hexane (HRR), ethyl acetate (ERR), chloroform (CRR), butanol (BRR), and aqueous extract (ARR), was studied against carbon tetrachloride (CCl4) induced hepato and testicular toxicity in rats (**Sahreen S., 2013**).
- The methanolic extract and its various fraction on the basis of polarity index were evaluated for phytochemical content, antimicrobial, cytotoxic and antitumor activities of extract from Rumex hastatus D. Don roots (**Sumaira Sahreen, 2013**).
- The essential oil was evaluated for anticholinesterase and antioxidant assays. The anticholinesterase assay was conducted at various concentrations (62.5 to 1000 µg/ml) against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Similarly,the antioxidant potential was determined using DPPH and ABTS free radicals **(Ahmad et al.2016)**.
- Anti-tumor and anti-angiogenic activities were carried out using potato-disc model and chorioallantoic membrane (CAM) assay respectively **(Ahmad *et al.2016*).**
- The methanolic extract and ethyl acetate extract of Rumex hastatus leaves were evaluated for the CCl_4 induced hepatic fibrosis **(Sahreen et al., 2017)**

AIM AND OBJECTIVE OF STUDY

The present study deals with the effect of *Rumex hastatus* leaves extract on Ethanol and Thioacetamide induced hepatic encephalopathy in rat. Prime objective of this work are following:

1. Evaluation of *in vitro* antioxidant activity of all successive extracts of *Rumex hastatus* leaves.
2. To analyze the protective effect of extract using serum preparation through biochemical estimations (SGOT, SGPT, Total bilirubin, albumin,alkaline phosphate)
3. To evaluate anti-inflammatory potential of Rumex hastatus extract by measuring the level of inflammatory markers like IL-1beta, TNF- alpha, NF-Kappa B.
4. To analyze the neuromodulatory properties of Rumex hastatus extract through the measurement of GABA, Glutamate.
5. To analyze the cholinesterase activity by using acetylcholinestrase kit.
6. To analyze the impact of Rumex hastatus extract using hepatic and brain tissue by performing histopathological studies.

PROPOSED RESEARCH METHODOLOGY

A. Collection and authentication of plant materials of *Rumex hastatus:*

Authentication of plants was done from Abhilashi Institute of Life Sciences, Tanda, Nerchowk. Mandi H.P.

- Voucher no. of *Rumex hastatus:*- AILS/1/RH/2011.

B. Extraction and Fractionation

The leaves of *Rumex hastatus* were dried in shade and coarsely powdered. Leaves will be successively extracted in the Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, methanol and water as solvent for the complete extraction of the phytochemicals. The five extract will dried in rota evaporator at 45 °C and the dried extracts are stored in vacuum desiccators containing anhydrous silica gel.

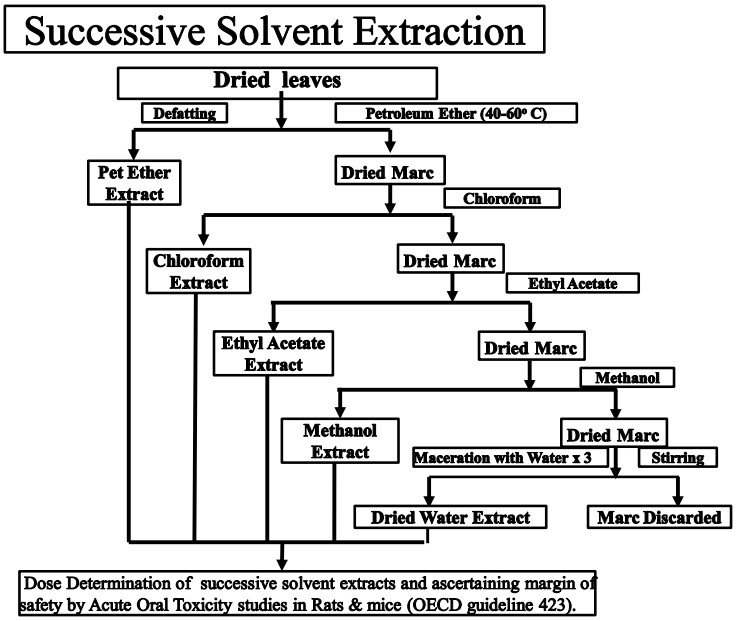

Fig.1: The complete scheme of the extraction and fractionation of dried leaves of *Rumex hastatus*

C. Protective effect of Rumex hastatus in liver cirrhosis by the following methods:

a) Ethanol induced hepatotoxicity.
b) Thioacetamide induced hepatotoxicity.

1. Ethanol induced hepatotoxicity in rats:

A total of 36 rats will be divided into 6 groups of 6 rats each**.(Samah A; 2017)**

- Group I served as control and received only the vehicle (1 ml/kg/day of 1% carboxy methyl cellulose (CMC); p.o.).
- Group II (positive control) received 40% ethanol p.o. once daily for 21 days.
- Group III received silymarin 25 mg/kg orally (p.o.) 1h before received 40% ethanol p.o. once daily for 21 days.
- Groups IV, V and VI were administered MERH at 100, 200, and 400 mg/kg body weight p.o. 1h before received 40% ethanol p.o. once daily for 21 days.

At the end of the experimental period (21 days), animals will fasted overnight and blood samples is obtained from the experimental and control rats by puncturing retro-orbital plexus. Collected blood is allowed to clot and serum will separated at 3000 rpm for 15 min for carrying out further biochemical investigations. One part of liver and brain will be dissected out and used for biochemical and histopathological studies.

2. Thioacetamide induced hepatotoxicity in rats:

A total of 36 rats will be divided into 6 groups of 6 rats each.

- Group I served as control and received only the vehicle (1 ml/kg/day of 1% carboxy methyl cellulose (CMC); p.o.).
- Group II (positive control) received TAA at 200mg/kg i.p. twice per week for four weeks. **(Somaya Z. Mansour et.al. 2016)**
- Group III received TAA at 200mg/kg i.p. twice per week followed by administration of silymarin 25 mg/kg p.o. daily for four weeks.

- Groups IV, V and VI were received TAA at 200mg/kg i.p. twice per week followed by administration of MERH at 100, 200, and 400 mg/kg body weight p.o. once daily for four weeks.

At the end of the experimental period, animals will fasted overnight and blood samples is obtained from the experimental and control rats by puncturing retro-orbital plexus. Collected blood is allowed to clot and serum will separated at 3000 rpm for 15 min for carrying out further biochemical investigations. One part of liver and brain will be dissected out and used for biochemical and histopathological studies.

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